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The Cadherins Fat and Dachsous Regulate Dorsal/Ventral Signaling in the *Drosophila* Eye

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Summary

The *Drosophila* eye is a polarized epithelium in which ommatidia of opposing chirality fall on opposite sides of the eye's midline, the equator [1–3]. The equator is established in at least two steps: photoreceptors R3 and R4 adopt their fates, and then ommatidia rotate clockwise or counterclockwise in accordance with the identity of these photoreceptors. We report the role of two cadherins, Fat (Ft) and Dachsous (Ds), in conveying the polarizing signal from the D/V midline in the *Drosophila* eye. In eyes lacking Ft, the midline is abolished. In *ft* and *ds* mutant clones, wild-type tissue rescues genetically mutant tissue at the clonal borders, giving rise to ectopic equators. These ectopic equators distort a mosaic analysis of these genes and led to the possible misinterpretation that *ft* and *ds* are required to specify the R3 and R4 cell fates, respectively [4]. Our interpretation of these data supports a significantly different model in which *ft* and *ds* are not necessarily required for fate determination. Rather, they are involved in long-range signaling during the formation of the equator, as defined by the presence of an organized arrangement of dorsal and ventral chiral ommatidial forms.

Results and Discussion

ft and *ds* Contribute to the Establishment of the Equator

ft has long been known for its role in proliferation control [5–7]. The identification of new alleles of *ft* in a FLP/FRT screen revealed a role for Ft in establishing epithelial polarity (D.J. Pan, personal communication; Figure 1). In ft^{422} null clones, approximately 52.5% of ommatidia—including mosaic and genetically mutant ommatidia—exhibit defects in polarity. Of these, 50.5% are inverted on their D/V axis (Figures 1A–1C). The remaining 2.0% of ommatidia are inverted on their A/P axis or on both their A/P and D/V axes. Furthermore, 98% of mosaic ommatidia that are phenotypically mutant are inverted on their D/V axis (1011 ommatidia scored from 22 ft^{422} clones).

Dorsoventrally inverted ommatidia are not randomly distributed within *ft* clones. Rather, they are preferentially localized toward the polar border such that the phenotypically mutant ommatidia are found in the polar region of the clone and phenotypically wild-type ommatidia are found along the equatorial border (Figures 1A–1C). The consequence of this biased distribution of ommatidia is an "inverted equator" (originally called a pseudoequator [8]) within the mutant clone, in which the points of opposing trapezoids face each other (Figures 1A–1C; white arrowheads). Inverted equators in *ft* clones consistently arise approximately two rows from the equatorial border of the clone. These inverted equators were seen in 35/41 (85%) ft^{422} clones. The 15% of *ft* clones with no apparent ectopic equator were either small or long and narrow and therefore not broad enough to detect this phenotype.

While a small percentage of *ft* clones lie along the equator (Figure 1C), of over 200 *ft* clones examined, none cross the equator. A closer examination of these clones revealed that the position of the endogenous equator, which can be identified in neighboring wild-type tissue, gets shifted by one to two ommatidial rows along the mutant border to accommodate the *ft* mutant clone (data not shown). These observations suggest the juxtaposition of ommatidia with high versus low Ft activity influences the placement of the equator.

We also show that *ds*, known for its role in morphogenesis [9], plays a role in setting up polarity in the eye (Figure 2). The bias of D/V:A/P errors is similar to that described for *ft* clones, although fewer ommatidia are disrupted. Approximately 29% of ommatidia (both mosaic and genetically mutant) in ds^{38K} clones (strong hypomorphic allele) display D/V inversions, while fewer than 1% of genetically mutant ommatidia display A/P inversions (517 ommatidia scored from 12 ds^{38k} clones; Figures 2A and 2B). Inverted equators also occur in *ds* mutant clones at a similar frequency as is seen with *ft*: 15 out of 18 clones (83%) had inverted equators (Figure 2A, black arrow). However, it is interesting to note that *ds* inverted equators arise along the equatorial border of the clone rather than within the clone, as is seen with *ft*. Finally, although ectopic *ds* equators are rare, they do occur (data not shown). This phenotype might be more penetrant in a null allele of Ds function; however, no such alleles have been reported.

Inverted and/or ectopic equators have also been observed in *mirr*, *fng*, and *four-jointed* (*fj*) [10–12]. One significant difference between *ft*/*ds* and these genes is that, in these other examples, mutant tissue nonautonomously disrupts wild-type tissue, generating a contiguous patch of nonautonomous D/V inversions [10, 11]. In *ft*, we have never seen nonautonomous effects on wild-type ommatidia. In *ds*, the nonautonomy can extend many rows beyond the clone and may be separated by up to eight rows of unaffected ommatidia. It is appealing to speculate that this "extended" nonautonomy is an effect of the twin-spot clone, in that a difference in relative amounts of Ds activity reverses the polarity. However, the scattered occurrence of these inverted ommatidia and the fact that we see a range in the number of affected ommatidia make this hypothesis somewhat unsatisfying. In order to test this "twin-spot" hypothesis unambiguously, again, a protein null allele of *ds* is necessary.

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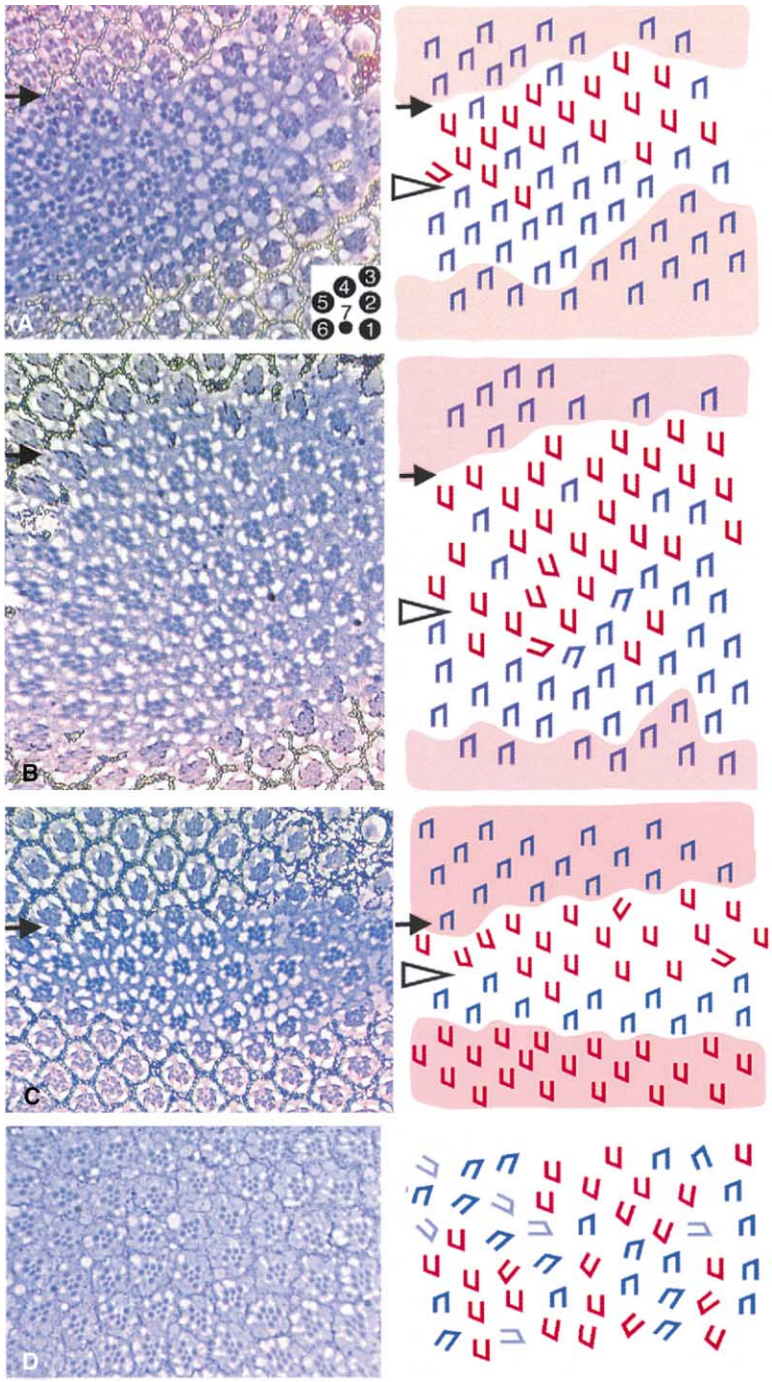

Figure 1. Ommatidial Polarity Is Disrupted in *ft* Mutant Clones

Seven of the eight rhabdomeres, the photosensitive portion of the photoreceptors, define a characteristic trapezoid shape in which the rhabdomere of R3 occupies the "point" of the trapezoid (inset [A]). Anterior/posterior (A/P) polarity is also present, such that photoreceptors R1, R2, and R3 define the anterior face of the trapezoid, and R4, R5, and R6 define the posterior face. (A–C) Tangential sections of adult hs-ft^{422} clones (left, marked by the absence of white pigment) and corresponding schematics (right, mutant tissue is white; wild-type tissue shaded red). Blue and red trapezoids represent the dorsal and ventral chiral forms of ommatidia, respectively. Light blue represents ommatidia that fail to rotate. Panels (A) and (B) show clones in the dorsal half of the eye; note the ectopic equator that forms along the polar border of the clone (black arrows). Blue trapezoids in clone include mosaic ommatidia. The clone shown in (C) lies on the equator. It is most likely that the lower equator is the endogenous equator since ectopic *ft* equators form along the polar border of the clone and endogenous equators respect the D/V boundary established at the equatorial border of *ft* clones (black arrow). Note the rescue of genetically mutant ommatidia along the equatorial border of the clone. This phenotype suggests Ft can act over a distance of two to four ommatidial rows to convey positional information. White arrowheads indicate "inverted equator." (D) An EGUF-ft^{422} eye (left) and corresponding schematic (right). Dorsal and ventral forms appear to be randomly distributed throughout the eye, leading to complete abolition of the endogenous equator. A significant percentage of ommatidia fail to rotate, a phenotype rarely seen in *ft* clones. (A–D) Anterior to the right.

Ft and Ds Participate in Global Signaling to Establish D/V Identity

The clonal phenotypes of *ft* and *ds* suggest that they are involved in establishing equators. Since the establishment of the equator is known to involve long-range signaling (reviewed in [13, 14]), the nonautonomous effects seen in the clonal phenotypes might be a consequence of a requirement for *ft* and *ds* in the transduction of a global patterning signal. To remove any effects of long-range signaling, we generated mutant eyes completely devoid of wild-type Ft using the *EGUF* system [15]. In contrast to *ft* clones, in *EGUF-ft* eyes, the D/V axis is so severely perturbed that the endogenous equator is abolished (Figure 1D). The greater degree of disruption observed in *EGUF-ft* eyes compared to mosaic clones suggests that wild-type tissue communicates with mutant tissue, perhaps via cell-cell relay of the signal transduced by Ft. If this is the case, then the presence of the inverted equator within the mutant clones is established as a consequence of signaling from wild-type tissue.

(The *EGUF* system does not affect polarity; however, a fraction of ommatidia are missing photoreceptors, but

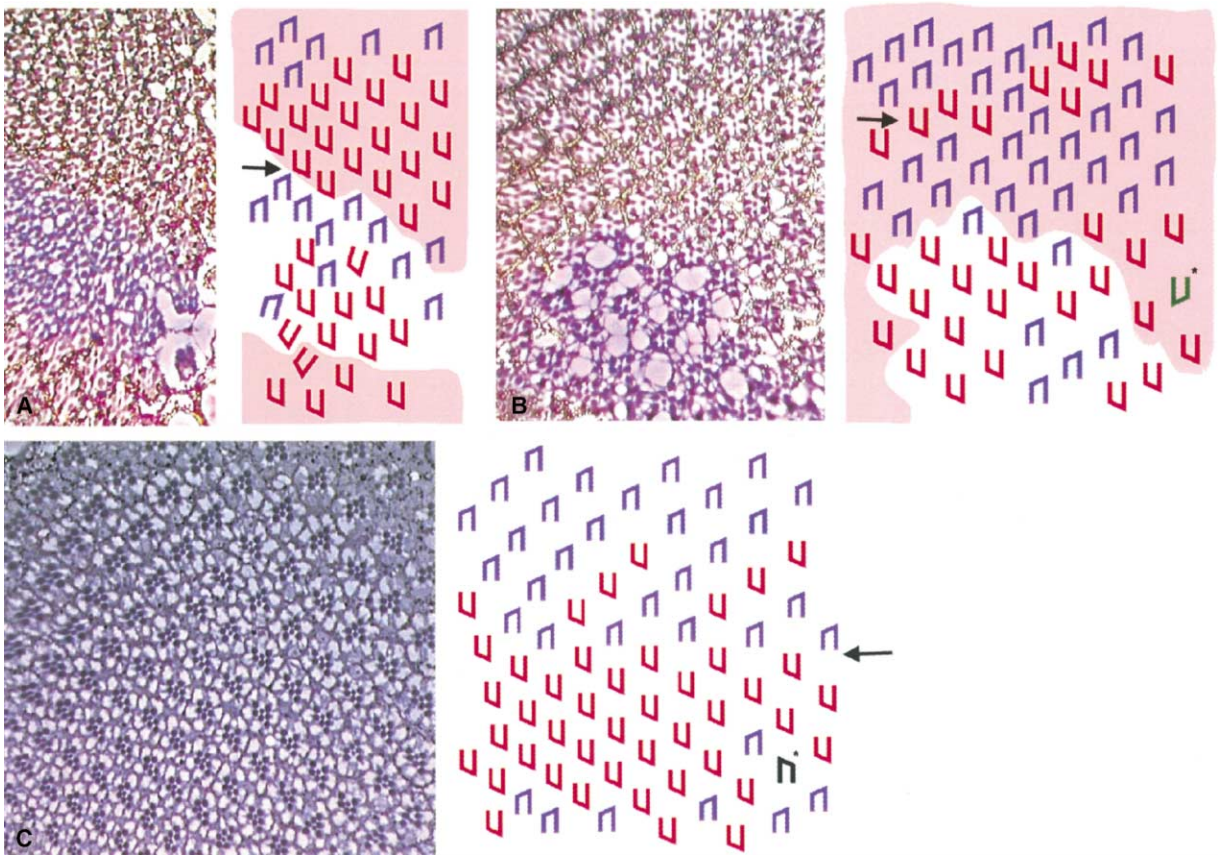

Figure 2. *ds* Mutant Clones Display Inverted Equators

(A and B) Tangential sections of adult hs-ds^{38k} clones. Corresponding schematics are shown to the right of each section.
(A) Inverted equator in the equatorial region of the clone (arrow).
(B) Nonautonomy occurs several rows outside of the clone (arrow). Green trapezoid, A/P inversion.
(C) Tangential section of an EGUF-ds^{38k} eye (endogenous equator indicated by arrow). Black trapezoid, AP/DV inversion.
(A–C) Anterior to the right.

this is an artifact of the *EGUF* system, as *EGUF*-wild-type eyes are missing similar numbers of photoreceptors. Furthermore, the *EGUF* system is leaky; only 2% of *EGUF-ft* flies survive.)

The *EGUF-ds* phenotype mimics the clonal *ds* phenotype: 31% of ommatidia display D/V inversions (Figure 2C). In *EGUF-ds* eyes, the endogenous equator is evident. This may be due to the weaker nature of this allele. Alternatively, it may suggest that Ds plays a more modulating role in establishing the D/V midline than does Ft.

Ft and Ds Regulate D/V Signaling Nonautonomously

Ft and Ds act nonautonomously in the eye. In *ft* clones, the majority of polarity defects occur in the polar region of the clone. In contrast, ommatidia in the equatorial region of the clone are phenotypically wild-type, suggesting that wild-type ommatidia outside the equatorial boundary of the clone rescue genetically mutant ommatidia within the equatorial region of the clone. Furthermore, wild-type tissue on the polar border of the clone does not rescue mutant ommatidia within the polar region of the clone, indicating rescue takes place only in an equatorial to polar direction and not from the poles to the equator. If this is the case, the Ft signal is propagated in a directional fashion from wild-type tissue at the equatorial border into the mutant clone. Finally, mutant tissue never nonautonomously affects wild-type tissue—in over 200 clones analyzed, we saw no inverted ommatidia in which all eight photoreceptors were wild-type. In contrast to *ft*, rescue takes place in a polar to equatorial direction in *ds* clones.

The tissue polarity genes *fz* and *stbm* are required to specify R3 and R4 [16, 17]. These cells then regulate the direction of ommatidial rotation. Given the importance of these two cells in the establishment of polarity, we examined ommatidia that were mosaic for *ft* within the R3 and R4 pair. In the majority of cases, the Ft^+ cell becomes R3 (Table 1). A similar analysis of the other developmental pairs of photoreceptors, R1/R6 and R2/R5, revealed that there is a strong tendency for the Ft^+ cell to adopt the fate of the anterior (R1 and R2) rather than the posterior (R5 and R6) photoreceptor cell (Table 1).

Table 1. R1, R2, and R3 Are Predominantly *fat*$^+$; R4, R5, and R6 Are Predominantly *ds*$^+$

	fat		*ds*	
Genotype in Mosaic Ommatidial Pairs	Percent	Number Mosaic Ommatidia Scored	Percent	Number Mosaic Ommatidia Scored
R1$^+$/R6$^-$	81	n = 96	27	n = 56
R1$^-$/R6$^+$	19		73	
R2$^+$/R5$^-$	75	n = 77	35	n = 37
R2$^-$/R5$^+$	25		65	
R3$^+$/R4$^-$	82	n = 116	23	n = 48
R3$^-$/R4$^+$	18		77	

There are two possible interpretations of these data. First, *ft* may be involved in specifying the anterior photoreceptor fates, as the data imply. However, if Ft is required to specify the R3 fate, we would expect to see at least a small fraction of ommatidia in which no R3 fate is specified in *ft* mutant tissue (i.e., ommatidia that have two R4s), as is the case in *fz* mutants [16], but we do not see this phenotype in *ft* clones. Alternatively, this finding could reflect the link between how cells are recruited into the growing ommatidium, how ommatidia rotate to establish polarity, and how this process is disrupted in *ft* mutant ommatidia, as explained below.

In doing this mosaic analysis, it was essential to recognize that a property inherent to eye development is that ommatidia that arise at the polar border of a clone predominantly recruit their polar cells from wild-type tissue and their equatorial cells from mutant tissue (see below; Figure 3A). Phenotypically mutant ommatidia occur only in the polar region of *ft* clones (Figures 1A–1C and 3B). This phenotype complicates the analysis and makes it difficult to draw conclusions regarding the specific cell(s) in which Ft is required for cell fate.

In wild-type clones, the cells that are recruited from the polar side of the clone (Figure 3: R4, R5, and R6 in both 1 and 4) will face the posterior (Figure 3: 1′ and 4′) side of the clone at the end of rotation. In *ft* clones, ommatidia that are both phenotypically mutant and mosaic occur only at the polar boundary of the clone (Figures 1A–1C). Since these ommatidia are almost always D/V inversions (Figure 3: 5′ and 8′), then they will have recruited their *ft*$^+$ cells from the polar side of the clone (Figure 3: 5 and 8), but rather than these polar-derived cells becoming posterior photoreceptors (R4, R5, and R6) as they would have in wild-type (Figure 3: 1,1′ and 4,4′), they become anterior photoreceptors (R1, R2, and R3; Figure 3: 5,5′ and 8,8′). Together, these factors create an artifactual bias in which the anterior photoreceptor cells are *ft*$^+$ and the posterior cells are *ft*$^-$ (Figure 3B). Consequently, even though the data appear to indicate *ft* is required for the anterior cell fates (R1, R2, R3), a more detailed analysis reveals that the nature of the *ft* phenotype introduces a developmental bias that must be considered.

The mosaic analysis of *ds* mutant clones revealed a trend opposite to that described for *ft*. In ommatidia mosaic for *ds* in the pairs R1/R6, R2/R5, and R3/R4, the majority of photoreceptors are wild-type for the posterior fates, R4, R5, and R6 (Table 1). Since D/V inversions are found on the opposite side of the clone in *ds* compared to *ft*, this is the expected result if one applies the same logic as described above for *ft*. As with *ft*, no functional autonomy can be assigned to a single cell.

A contrasting interpretation of a mosaic analysis of *ft* and *ds*, presented by Yang et al. [4], suggests *ft* and *ds* are required to specify the fates of photoreceptors R3 and R4, respectively. However, mosaic analyses of *ft* and *ds* are inherently biased due to the clonal phenotypes, as described above. This bias might mask a role

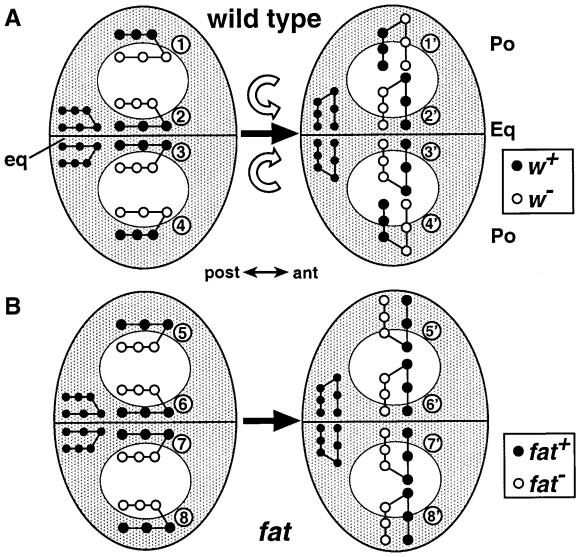

Figure 3. Recruitment of Cells in Mosaic Eyes and Subsequent Ommatidial Rotation

Wild-type tissue shaded with black dots; clones shown as white ovals. Two eyes are shown for each genotype: the left eye shows ommatidia prior to rotation, and the right eye is postrotation. Each circle within the trapezoid represents a photoreceptor, and the color indicates the tissue from which it was derived: black photoreceptors were recruited from wild-type tissue, and white photoreceptors were recruited from the clone. (A) Clones for wild-type are marked by the absence of a color marker (*w*) to allow the reader to discriminate clonal from nonclonal tissue. In wild-type, ommatidia in the dorsal half of the eye rotate counterclockwise, and, in the ventral half, they rotate clockwise (arrows). (B) *fat* mutant clone. See text for details. The *ft* mosaic analysis included 289 mosaic ommatidia, both phenotypically mutant and wild-type, 114 of which had informative R8 data. Since mosaic ommatidia for which R8 could not be scored are consistent with the above 114 ommatidia, therefore all 270 mosaic ommatidia were included. Anterior to the right. Eq, equatorial; Po, polar; *w*$^+$, wild-type tissue, marked with pigment (black circles); *w*$^-$, clonal tissue, lacking pigment (white circles); post, posterior; ant, anterior; eq, equator.

for *ft* or *ds* in the R3/R4 fate decision, but currently there is no compelling evidence for such a functional requirement. The genetic data reported here and in Yang et al. [4] are insufficient to draw conclusions about the role of *ft* and *ds* in fate specification. Extensive experimentation and a better understanding of mechanism are necessary to discriminate between a role for *ft* in global D/V signaling versus a requirement for *ft* and *ds* in specification of the R3 and R4 cell fates.

ft Acts Downstream or in Parallel to *mirr*

Early acting genes, for example, *mirror* (*mirr*) and *fringe* (*fng*), specify the dorsal and ventral halves of the eye, respectively, thereby setting up the D/V boundary (reviewed in [3]). The *ft* clonal phenotype suggested *ft* might mediate D/V boundary formation. To address this possibility, we assessed the effect of *ft* on *mirr* expression. The expression pattern of the enhancer trap line 8A5, in which the *mirr* promoter drives expression of *white*, is unaffected in EGUF-*ft* eyes. In these eyes, *mirr* expression remains restricted to the dorsal half of the eye, indicating that *ft* is required downstream or in parallel to *mirr* (see the Supplementary Material).

ft and *ds* Interact with Tissue Polarity Genes

Given that *stbm* interprets D/V signals and *fmi* encodes a Fat-like cadherin, we tested for genetic interactions between *ft* and *ds* and these genes. (See the Supplementary Material available with this article online.)

A Model for *ft* Activity in the Eye

Our model for *ft* activity (Figure S4) differs from that of Yang et al. [4] because we (1) take into account the observations that ectopic equators are generated in mutant clones and that the *ft* clonal phenotype differs significantly from the *EGUF-ft* phenotype and (2) consider fundamental properties of eye development in conjunction with the clonal phenotype. We propose a model in which *ft* conveys D/V positional information to developing ommatidia to create the D/V midline.

Ft functions to inhibit D/V signaling in the wing and haltere [6, 18]. Our data are consistent with this proposal—new equators are generated in *ft* mutant clones in the eye. We propose that a consistent level of Ft activity throughout the eye inhibits the D/V signaling required to form the equator. At the equator, Ft activity must be inhibited. The molecule that inhibits Ft could be expressed in several ommatidial rows encompassing the future midline. In a *ft* mutant clone, the phenotype is rescued for two rows in the equatorial region of the clone, suggesting the D/V signal propagated by Ft can be relayed for a distance of two ommatidial rows. Therefore, we propose that in the presence of the regulatory protein, the D/V signal can also be relayed an equivalent distance at the endogenous equator. When the D/V signal reaches its minimum, tissue with Ft activity apposes tissue without Ft activity, and it is at this point that the equator is established.

Supplementary Material

Supplementary Material including a supplementary Results and Discussion section, four figures (Figure S1, Ommatidial Polarity in Wild-Type *Drosophila* Retina; Figure S2, *fat* and *dachsous* Interact Genetically with *sev-stbm*; Figure S3, Wild-Type *mirr* Expression in *ft* Eyes Indicates *ft* Is Downstream of *mirr*; and Figure S4, A Model for *ft* Activity in the Developing Eye), and a table (Table S1, *fat* and *ds* Show Genetic Interactions with Other Tissue Polarity Genes) can be found at http://images.cellpress.com/supmat/supmatin.htm.

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